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THE QUANTITATIVE DETERMINATION OF C^{14} ACTIVITY IN BIOLOGICAL SYSTEMS BY DIRECT PLATING

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John R. Hogness
Lloyd J. Roth
Edgar Leifer
Wright Langham

Los Alamos Scientific Laboratory

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THE QUANTITATIVE DETERMINATION OF C^{14} ACTIVITY IN BIOLOGICAL SYSTEMS BY DIRECT PLATING

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With the increased use of C^{14} tagged compounds in biological tracer studies a method for direct and rapid quantitative determination of radioactivity in biological fluids becomes important.

One satisfactory but tedious method involves combustion of the organic material in such fluids as blood, plasma, and urine, collection of the CO_2 and the precipitation on suitable plates as $BaCO_3$, according to the method described by Yankwich.[1] This method implies that the C^{14} containing compound under study be similarly assayed by combustion and counting as $BaCO_3$.

A more rapid method involves the direct plating of aliquots of the biological fluids themselves onto suitable counting plates and subsequent determination of radioactivity with a thin mica window (Geiger Mueller) tube. The latter method has been used in some of our studies of the metabolism of nicotinic acid and related compounds. It became apparent, however, that for valid interpretation of the measured activity obtained in this manner, suitable calibration is essential.

METHOD

In preparing calibration curves, increasing amounts of urine, plasma, or laked red cells were added to a series of tubes, each containing a known constant amount of a water solution of radioactive nicotinic acid (C^{14} in the carboxyl group)[2] The contents of each tube were thoroughly mixed and quantitatively transferred to copper discs (oxidized surface) which were rotated on a horizontal turntable during plating. The plates were dried with an infrared lamp and hot air blower, and counted using a GM tube with a thin mica window weighing 1.7 mg/cm^2 and having a diameter of 5.4 cm. The circular area plated on the copper disc was 16 cm^2. The response of this GM tube when using discs of this size was evaluated by plating a water solution of radioactive nicotinic acid (specific activity = 75,000 c/sec/mg) at different points and over a series of increasing areas of the copper plate. The results obtained were reproducible within the inherent error of the counting system if plating was within an area of 16 cm^2.

DISCUSSION

Since the effects noted were similar and reproducible for all biological fluids studied (urine, plasma, laked erythrocytes), the results presented in Figure 1 are representative.

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[1]Yankwich, Peter E., et al., Ind. and Eng. Chem., Anal. Ed. 19:439–441 (1947).

[2]Murray, Arthur, W. W. Foreman, and Wright Langham, Science 106:277 (1947).

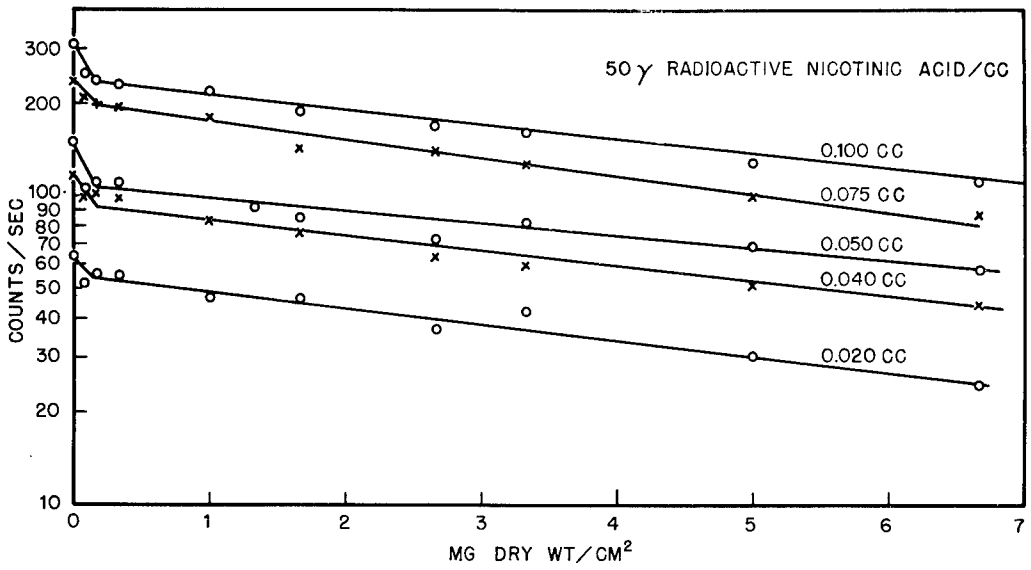

Figure 1. Urine calibration curves; constant radioactivity with increasing amounts of urine.

The data, plotted on semilog scale, indicate that the addition of very small amounts $\frac{(0.025\ cc)}{(0.08\ mg/cm)}$ of urine to a constant amount of radioactivity produces an initial decrease measured activity. This drop amounts to about 20 % of the original activity determined by plating and counting a water solution of nicotinic acid containing C^{14} to which no urine has been added. Additional plates made by the above method, using increasing amounts of urine, show a constant decrease in measured activity consistent with the anticipated absorption curve for weak beta rays. A similar effect was noted when varying amounts of saline were substituted for biological fluids.

It, therefore, becomes apparent that when using the direct plating method for the determination of radioactivity, it is imperative that calibration curves be made for all biological fluids used and that the thickness of the plates be such as to fall on the second portion of the curve. By using such calibration curves and interpolating back to the ordinate, we have been able to account for better than 90 % of the original activity in a system of in vitro biological studies as well as in vivo metabolism experiments.

In an attempt to evaluate this phenomenon, all plates were examined microscopically. Those plates representing the activity obtained for the second portion of the curve exhibited a uniform distribution of fine crystals in an amorphous matrix. However, those plates representing the initial portion of the curve, in which the slope is greatest, showed a less uniform distribution of fewer but larger crystals. This difference is shown in the accompanying photomicrographs (Plates I and II).* The fluctuations in the initial portion of the curve may be accounted for by the inhomogeneity of the material on the plates at these low thicknesses.

*(These plates were not submitted with manuscript for reproduction.–A.E.C., T.I.D.)

Since backscattering is a function of the composition of the background plate as well as the beta ray energy spectrum, it is necessary that the same material be used for the plates in calibration and in recurring experimental data.

Experiments to evaluate backscattering by oxidized copper surfaces in our system show that 25 % of the measured activity is due to backscattering when a water solution containing radioactive nicotinic acid, resulting in an infinitely thin layer, was plated.

Backscattered electrons have considerable lower energies than the average C^{14} beta ray. It seems likely that the initial increase in activity noted (20%) is due to absorption of the low energy beta rays (25%) by the material present in biological fluids, which are precipitated with the radioactive compound.

In order to demonstrate that this phenomenon is related to low energy beta emitters, an experiment was carried out using P^{32} (maximum energy of beta particles, 2 Mev, as compared with 0.14 Mev for C^{14}) and the effect reported in the preceding paragraph was not noted in plates of similar thickness. This implies that this effect is specific for low energy beta ray emitters.

SUMMARY

An effect which is essential in the determination of radioactivity by direct plating of biological materials containing low energy beta ray emitters (C^{14}) is reported.

The use of calibration curves such as those presented is necessary if quantitative recoveries are to be obtained using direct plating methods.

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